IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant:	YAMANOUCHI, Masaya et al.	Conf:
Appl. No.:	09/578,693	Group: 1641
Filed:	May 26, 2000	Examiner: L. Cook
For:	METHOD FOR EXAMINING HUMAN KIDNEY DISEASE BY DETECTING THE FATTY ACID BINDING PROTEIN	

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Takeshi SUGAYA a citizen of Japan residing at Itami-shi, Hyogo-ken, Japan, hereby declare as follows:

1. I am a co-inventor of the subject matter of the above-referenced patent application.

2. I graduated from the Faculty of Engineering of Kyoto University, Japan in March 1986. I also received my Masters degree in Engineering from Kyoto University in March 1989 and my Ph.D from the University of Tsukuba in November 1999.

3. From April 1989 to date, I have been employed by Tanabe Seiyaku Co., Ltd., Osaka, Japan, the assignee of the above-identified application. From April 1989 until October 2000, I was engaged in research works in the fields of biochemistry and pharmacology at research laboratories of Tanabe Seiyaku Co., Ltd. From November 2000 to present, I have been was on loan to CMIC Co., Ltd., Tokyo, Japan, an outsourcing pharmaceutical development company, one of the Contract Research Organizations (CROs). At CMIC Co., Ltd., I have been engaged in research and development of diagnostic reagents, and methods and systems including the same in the field of diagnosis of renal disease directed to L-FABP. My present position is as the project leader of business development at CMIC Co., Ltd.

4. I am the co-author of about 68 papers in the field of cardiovascular and nephrology, and a co-author of about 11 papers in the field of diagnosis of renal disease focused on L-FABP. I received the young investigator award from the Society of Japan Cardiovascular Endocrinology and Metabolism in 1997. Since January 2005 to present, I was delegated as a guest professor at the department of Nephrology and Hypertension at St. Marianna University School of Medicine, Japan.

5. I have read and understood the contents of U.S. patent application no.: 09/578,693 and am familiar with the prosecution history of said application.

6. Under my direction, the following experiment has been done.

[I] Object of the experiment:

The following experiment has been done in order to prove that the method of the present invention in which L-FABP is detected as a marker show superior effectiveness over a method in which H-FABP is detected, and to prove that L-FABP and H-FABP are not similar and/or equivalent, and H-FABP is not an obvious substitute for L-FABP.

[II] Methods:

(1) Subjects:

Urine samples were collected from seven human individuals having moderate kidney dysfunction and who underwent radiographic examination procedures (coronary angiography) accompanied by administration of contrast media.

The individuals were those with serum creatinine level greater than normal range(*) [5 men and 2 women; mean age, 66 years old; mean value of the serum creatinine level were 1.31mg/dL in men and 1.05mg/dL in women]. [*: upper limit of normal range in the hospital was 1.04 mg/dL in men and 0.79 mg/dL in women].
In the study, individuals with the following were not included: end-stage renal disease under hemodialysis, liver disease and cancer.

The contrast media used was a non-ionic low-osmolarity type agent.

Before and 8, 16, 24 and 48 hours after administration of the contrast media, the urine samples were collected from each individuals, and the level of FABPs (L-FABP

and H-FABP) in the urine samples were measured. The concentration of FABPs in the samples was measured as described in the following (2) and (3).
The concentration of creatinine in the samples was measured by conventional enzymatic method. The value of FABP concentration was corrected by the value of urinary creatinine concentration to be shown as the level of urinary FABP (μg/ g creatinine).

(2) L-FABP assay:

L-FABP in urine sample (urinary L-FABP) was measured by sandwich ELISA (Enzyme-linked immuneoabsorbent assay).
The assay was carried out by using a kit containing monoclonal antibodies specific to human L-FABP (human L-FABP ELISA kit; CMIC Co., Ltd.(Japan)), and following the detailed instructions described in the manual of the kit.

Briefly, samples treated with pretreatment solution were transferred into anti-L-FABP antibody immobilized microplate containing assay diluent.
After incubation, the microplate was washed and added with a reagent containing peroxidase-conjugated secondary antibody. After incubation, the microplate was washed and added with substrate for enzyme reaction. After reaction, the optical density was measured using a microplate reader, and calibration curve was prepared based on the obtained optical density, thereby determining the L-FABP concentration.

(3) H-FABP assay:

H-FABP in urine sample (urinary H-FABP) was also measured by sandwich ELISA as in the above (2). The assay was carried out by using a kit containing monoclonal antibodies specific to human H-FABP (human H-FABP ELISA kit; HyCult biotechnology b.v. (Netherlands)), and following the detailed instructions described in the manual of the kit.

Breifly, samples were transferred into anti-H-FABP antibody-immobilized microplate containing assay diluent. Thereto were added a reagent containing peroxidase-conjugated secondary antibody. After incubation, the microplate was washed and added with substrate for enzyme reaction. After reaction, the optical density was measured using a microplate reader, and calibration curve was prepared based on the obtained optical density, thereby determining the H-FABP concentration.

[III] Results:

It is known that administration of contrast media used in radiographic examination procedures is a load/stress for kidney and cause induction of renal injury.

In this study, before and after administration of the contrastmedia to individuals who underwent radiographic examination procedure, the urine samples were collected and the level of L-FABP were measured to study the change with lapse of time to monitor the renal injury induced by administration of contrast media.

In addition, as a comparative study, the level of H-FABP in the same samples were measured.

The results are shown in Table 1, and the same is graphed out in Figure 1.

As shown in Table 1 and Figure 1, the level of urinary L-FABP has distinctly elevated with lapse of time after administration of contrast media. However, such was not observed in the level of urinary H-FABP. The level of urinary H-FABP hardly changed and was at a low level throughout the course of time.

The results of this study show that the renal injury induced by contrast media can be sensitively examined by measuring the level of L-FABP, however, it cannot be done as well by measuring H-FABP instead of L-FABP.

Table 1

	Urinary FABP (μg/g creatinine) Mean±S.D.				
	Pre*	8 hr **	16 hr **	24 hr **	48 hr **
L-FABP	4.24±2.98	11.58±12.95	30.12±23.02	44.68±18.62	26.64±25.45
H-FABP	0.08±0.20	0.71±0.94	0.73±1.16	1.17±1.48	0.11±0.29

*: before administration of contrast media
**: time after administration of contrast media

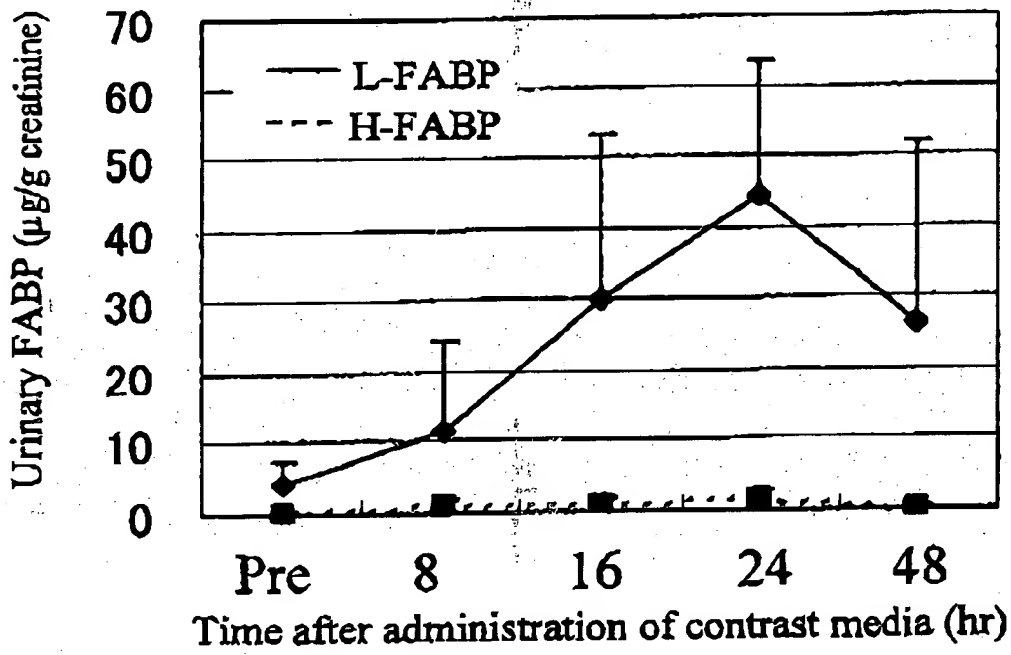

Fig. 1

7. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

Signed this day 14 of March, 2006

Takeshi Sugaya

Takeshi SUGAYA